Assessing Developmental Competence through miRNA Markers in Aneuploid Human Blastoids

Zuleika C. L. Leung[1]; Allison H. Y. Tse[1]; Dean H. Betts[1, 2, 3]

1. Department of Physiology & Pharmacology, Schulich School of Medicine & Dentistry, University of Western Ontario, London ON, Canada
2. Department of Obstetrics & Gynaecology, Schulich School of Medicine & Dentistry, University of Western Ontario, London ON, Canada
3. Children's Health Research Institute (CHRI), Lawson Health Research Institute, London ON, Canada

The Centers for Disease Control and Prevention's 2020 report on Assisted Reproductive Technology (ART) revealed that only 45% of embryo transfer procedures result in live birth deliveries. The current criteria for embryo transfer rely on simple evaluations like grading of morphology and timing of compaction. However, a more thorough evaluation of an embryo's status using preimplantation genetic testing for aneuploidy (PGT-A) requires the biopsy of a trophectoderm cell(s), and this method overlooks the possibility of genetic mosaicism in an embryo.

Aneuploidy is a frequent cause of embryo developmental failure during the pre-implantation stages. Studies have found unique miRNA markers in different trisomy cell lines. According to recent research, these miRNA markers show potential as predictors of successful implantation. MicroRNAs (miRNAs) released by mammalian embryos into the culture media could be important, unbiased, and non-invasive markers for evaluating embryo quality. Unfortunately, using human embryos as a research model is challenging due to sample limitations and ethical concerns.

Our laboratory has established a blastoid protocol that models human blastocyst development using pluripotent naïve-like H9 human embryonic stem cells (hESCs). By inhibiting the Hippo, TGF-β, and ERK pathways in aggregated naïve-like hESCs, we efficiently generated blastoids that resemble human blastocysts. Blastoids present a fluid-filled cavity and the self-organization of three primary cell lineages. We evaluated the presence of all three primary lineages and their proportions using RT-qPCR and immunofluorescence microscopy. $n > 3$.

To induce aneuploidy before blastoid generation, we treated hESCs with 0.5 uM reversine, a spindle assembly checkpoint inhibitor, for 24 hours. miRNA markers with links to developmental competence (e.g. miR-191, miR-320a, and miR-372) were evaluated in blastoids and their spent culture media fractions using RT-qPCR. Unlike non-treated controls, we observed a much lower percentage of cavitation in reversine-treated blastoids. We also identified a higher frequency of cell death in blastoids exposed to reversine treatment. Elevated levels of miR-320a and miR-372 were found in the spent culture media fraction of reversine-treated blastoids compared to non-treated blastoids.

Overall, our study presents blastoids as a plausible and reflective human embryo model. These results provide insights into identifying candidate miRNA markers of developmental competency via blastoids generated from hESCs of known aneuploid status.